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THE FOLLICLE SACS OF THE AMPHIBIAN OVARY.

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In 1738, Swammerdam (6) stated that just after the eggs of the frog have been laid, one can find on the inner wall of the ovary, "some empty and very delicate membranes which had served to invest the eggs that had been already discharged from the ovary. . . . These particles were most beautifully interwoven with blood vessels to which they were fixed as to so many stalks." Swammerdam does not give the origin of these membranes nor does he state how the eggs pass from the ovary into the body cavity.

Over one hundred years later, Thompson (7) wrote that the ovarian egg of the frog and toad "is surrounded by a thin vascular sac formed by the dilation of the ovisacs which hang into the general ovarian cavity. This capsule or ovisac is attached to the rest of the ovarian substance by a broad band rather than by a narrow pedicle; and when the yolk or ovarian ovum is mature, it escapes from the ovisac by the formation of an aperture in the remote or free side of this capsule, somewhat in the same manner as occurs in the calyces of the bird, but with a wider aperture. Through the apertures of the general ovarian capsule the numerous ova pass into the abdominal cavity." In thus stating that the ovarian eggs first fall into the general cavity of the ovary and later pass into the body cavity through openings in the ovarian wall, Thompson is in agreement with earlier writers, Rathke (4) and Lereboullet (3). More recently this view has been opposed by Brandt (1) who examined the outer surface of the ovary of *Rana temporaria* as the eggs were about to pass into the body cavity and found a round hole above each egg through which a larger or smaller part of the egg protruded. This discovery led him to state: "Es ist mithin klar, dass für jedes sich lösende Ei

eine besondere Austrittsöffnung in die Peritonealhöhle hergestellt wird."

According to Brandt, the ovarian egg of *Rana* is surrounded by membranes whose origin he describes as follows: "Die Follikel des Ovariums dürften dadurch entstehen, dass die einzelnen sich vergrössernden Eianlagen von den benachbarten, sich proliferirenden und abplattenden Elementen, sowie von den Bindegewebselementen der Ovarialwandung umwachsen werden." These membranes are said to form the follicle sacs that are found on the inner wall of the ovary after the eggs have passed into the body cavity, but no mention is made of their later history. During his investigations, Brandt carefully injected colored fluids into the ovary just after the eggs had been laid and found that none of the fluid ever came through the walls of the follicle sacs. He was thus able to show conclusively that there is no communication between the cavity of the ovary and the body cavity through the walls of the follicle sacs. The latter have but the one opening which leads outward into the body cavity.

The investigations recorded in the present paper were made to supplement the work of Brandt and others with a more detailed description of the origin and fate of the follicle sacs of the amphibian ovary. Observations were made on three different amphibians, *Bufo lentiginosus*, *Rana palustris* and *Hyla*. The general description and the drawings (made with a camera lucida) are taken entirely from *Bufo lentiginosus* as I had a much more complete series of this species. As the relatively few stages of *Rana* and *Hyla* at my command confirmed the results obtained on *Bufo* in every respect, a separate description of the follicle sacs in these two amphibians seemed unnecessary and is, therefore, omitted. In preparing the material for study, small portions of the ovary were fixed in corrosive-acetic, and then stained on the slide either with iron-hæmatoxylin, or with a mixture of Lyon's blue and borax carmine, as described in a previous paper (King, 2).

The ovary of *Bufo lentiginosus* consists of several distinct compartments, agreeing in this respect with the ovary of various species of frogs, according to the observations of Rathke, Lereboullet, Brandt and Schultze (5). The walls of these compart-

ments are made up of two membranes which, as a rule, are separated some distance, although occasionally they lie so close together that they appear to be completely united. The outer membrane is single, compact, and of nearly uniform thickness throughout the entire ovary. The inner membrane, on the contrary, appears in some places to be very similar in structure to the outer one; in other parts of the ovary it is found to be composed of two thin layers which always lie close together and are connected at frequent intervals. Cells with very large nuclei are closely applied to the outer and inner surfaces of these membranes and are also present in the spaces between them. (Figs. 3–6, *O. W.*)

The eggs develop between the two membranes composing the wall of the ovary. As they increase in size, they press against the inner membrane of the ovarian wall and cause it to project more and more into the cavity of the ovary. Part of the ovarian

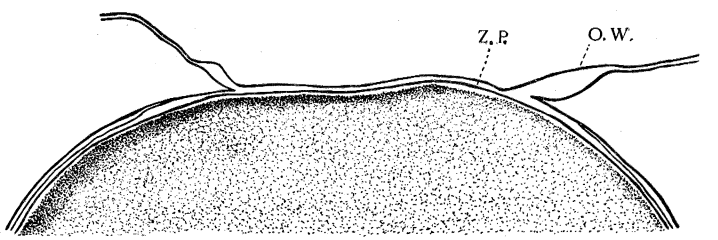

FIG. 1. A portion of an ovarian egg showing the manner of its attachment to the wall of the ovary. Z. P. Zona pellucida; O. W-, outer wall of the ovary. Zeiss. obj. A. Oc. 4.

wall comes, therefore, to form a sac that incloses the egg and supplies it with nourishment as long as it remains in the ovary. When the egg is still quite small, there is formed, close to its outer surface, a true egg-membrane, the "zona pellucida" or "chorion," which is thick and seemingly homogeneous in structure (Fig. 2, *Z. P.*). This membrane which is formed, probably, by the numerous follicle cells that are scattered all over the surface of the egg, surrounds the egg during its growth and maturation periods and is invariably found around all eggs taken from the body cavity. Numerous cells and occasionally blood corpuscles (Fig. 2, *C.*) are found between and under the two layers

of membrane which form the outer covering of the egg. These cells, which are usually oblong in shape, contain a small amount of faintly staining, granular protoplasm, and a very large, rounded nucleus with one nucleolus. The chromatin is either collected in masses along the nuclear wall, or it is in the form of a more or less regular network scattered throughout the nucleus.

Ordinarily, when the egg of *Bufo lentiginosus* is ready to leave the ovary, it has a diameter of about 1.5 mm. and from 0.60–0.70 mm. of its surface is in contact with the outer membrane of the ovarian wall (Fig. 1). In the great majority of cases, the egg is attached at the equatorial region as in Fig. 1; but it is not uncommon to find among the eggs thus attached, one with its black or its white pole against the outer membrane.

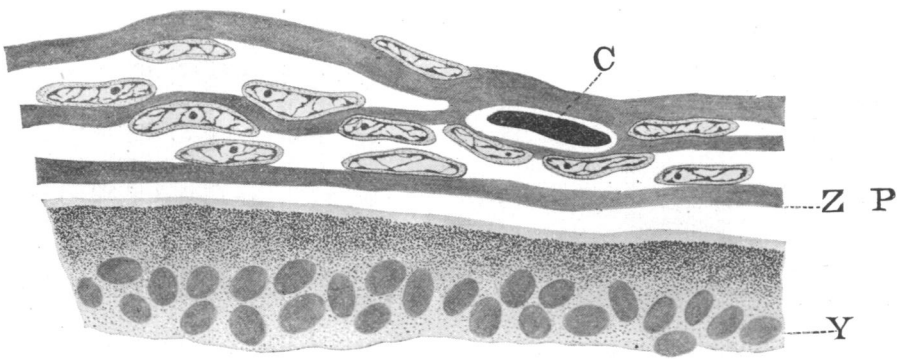

FIG. 2. Part of an ovarian egg highly magnified, showing the structure of the membranes surrounding the egg. C, blood corpuscle; Y, yolk; Z.P., Zona pellucida. Zeiss apoc. 2 mm. Oc. 8.

By what means the ovarian wall is ruptured and the eggs set free into the body cavity is not known. This process cannot be connected with the breaking down of the germinal vesicle, because the nucleus degenerates and the first polar spindle is formed some time before the egg leaves the ovary, as I have shown in a previous paper (2); neither can it be due to the way in which the eggs are attached to the ovarian wall, for, as I have stated above, the mode of attachment varies with individual eggs. Apparently the eggs must reach a definite stage of development before they break away from the wall of the ovary, because, although I have

sectioned a large number of eggs taken from the body cavity, I have never found a single one in which the polar spindle was not fully formed with the chromosomes arranged along the spindle fibers. Brandt suggests that there may be an active con-

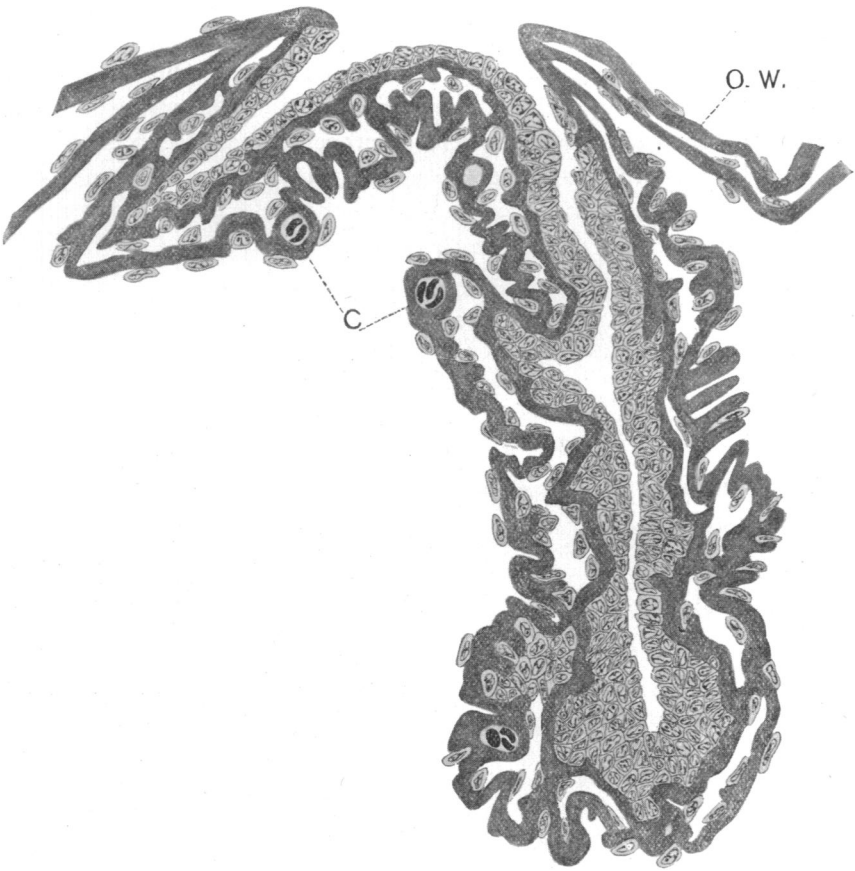

FIG. 3. Longitudinal section of a follicle sac just after the egg has passed into the body cavity. C, blood corpuscles; O. W., ovary wall. Zeiss obj., D. Oc. 4.

traction of the follicle sac when the egg is ready to leave the ovary, and that this contraction stretches the base of the follicle and causes a rupture. How such a contraction takes place without the presence of muscles in the walls of the follicle sac is not at all clear. It seems to me that the rupture of the ovarian wall is more likely to be due to some activity on the part of the egg

itself. Possibly there is a slight increase in the size of the egg at this time which produces sufficient pressure on the surrounding membranes to cause a break at the weakest point. This would seem to be where the egg is attached, because here there are only two membranes over the egg instead of three (Fig. 1). This suggestion, however, fails to account for the rare cases where the egg breaks through its membranes and falls into the cavity of the ovary. Such eggs never leave the ovary; sooner or later they undergo degenerative processes and are gradually absorbed, as are also the few eggs that do not leave the mem-

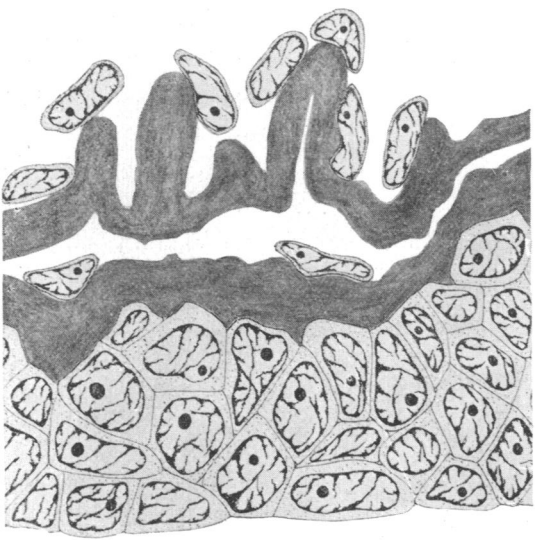

FIG. 4. Part of the follicle sac shown in Fig. 3 more highly magnified. Zeiss apoc. 2 mm. Oc. 8.

branes at all but remain attached to the ovarian wall. In the latter case the eggs are almost invariably smaller than those taken from the body cavity, and, therefore, retarded development may explain their failure to undergo normal processes.

On spreading out a piece of the collapsed ovary after the eggs have passed into the body cavity, one can plainly see the numerous follicle sacs without the aid of a microscope. These sacs vary in length from 0.36 mm. to 0.54 mm., and their walls are very much wrinkled and folded. Although from 0.60 to 0.70 of

the mature ovarian egg is in contact with the outer membrane of the wall of the ovary, I have never found the opening of an empty follicle sac to be more than 0.38 mm., the average being 0.20 mm. This shows that the edges of the opening tend to close in as soon as the egg has passed out of the ovary.

On examining a longitudinal section of an empty follicle sac (Fig. 3), one finds that the two layers of which its walls are composed are united at frequent intervals, and that, on the wall and in the spaces between the layers, there are numerous cells.

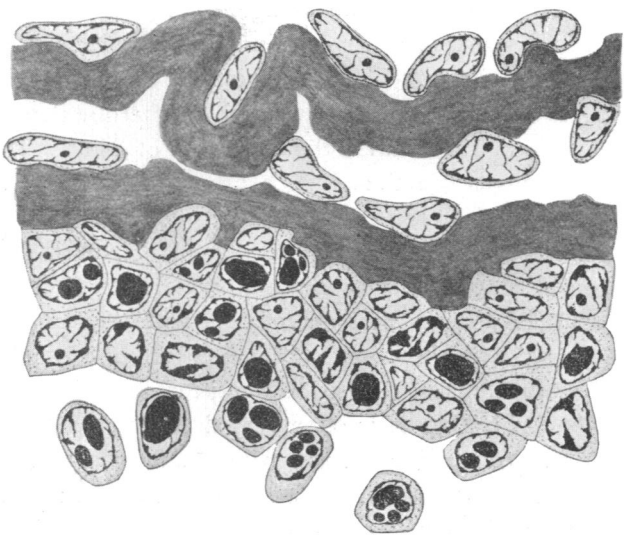

FIG. 5. A portion of the wall of a follicle sac after degenerative changes have begun. Zeiss. apoc. 2 mm. Oc. 8.

Sections of the small blood vessels which supplied the eggs with nourishment are also found occasionally (Fig. 3, *C*). The cavity of the follicle sac is lined with cells, several layers deep in places, particularly at the closed end of the sac. These cells are doubtless the ones that lay between the zona pellucida and the outer covering of the egg at an earlier period, and their confinement in a much smaller space must explain why they appear so numerous, as I have never found any evidence of cell division at this time.

A part of the wall of a follicle sac more highly magnified is shown in Fig. 4. The cells lining the cavity of the sac are seen

to be very closely packed together, and there is rarely an intercellular space to be found. These cells have the same characteristics as those shown in Fig. 2; namely, a very large nucleus containing one nucleolus, a small amount of granular protoplasm, and chromatin partly in the form of a network, and partly in masses scattered along the wall of the nucleus.

On examining the ovary of a toad killed about three weeks after the eggs had been laid, one finds that the openings of the follicle sacs into the body cavity have closed, and that the outer membrane of the ovarian wall is again continuous. Each follicle sac appears at this time as a blind pouch projecting from the inner side of the ovary. The cells lining the cavity of the sac are beginning to show the degenerative changes that are very marked in the ovaries of toads killed the latter part of May. The most noticeable changes are those which are taking place in the nucleus (Fig. 5). Although at this time the nucleus is still round or oval, its outline is somewhat more irregular than it was at an earlier period, and most of its chromatin is collected in from one to four rounded masses that stain black with iron-hæmatoxylin and a deep red with the borax carmine and Lyon's blue mixture used. The protoplasm of the cells appears very granular, and it stains so feebly that it is often very difficult to determine the cell outlines. Such changes, as a rule, are first apparent in the innermost cells that soon break away from the rest and become scattered throughout the cavity of the follicle sac. These free cells never show any pseudopodia; on the contrary, they are more rounded than are the cells still attached to the walls of the follicle sac, and it is therefore impossible to determine whether they have the power of independent movement or not.

Although I have examined the ovaries of many toads killed during the month of June, I have not been able to determine whether the cells lining the follicle sacs are destroyed by the leucocytes, as are the eggs that fail to leave the ovary, or whether they disintegrate and are absorbed by the cells of the ovarian wall. In all the toads killed at this time, either the cells within the follicle sacs were undergoing degenerative changes similar to those shown in Fig. 5, or else the cavity of the follicle sac was nearly obliterated and no traces of the cells could be found. It

is evident that the final stages in the disappearance of these cells take place very quickly and that a large amount of material would be necessary in order to trace their entire history. A longitudinal section of one of the follicle sacs found in the ovary of a toad killed on the fifth of June is represented in Fig. 6. The sac measures 0.13 mm. in length and is, therefore, about one third its original length. The walls of the sac are thinner and

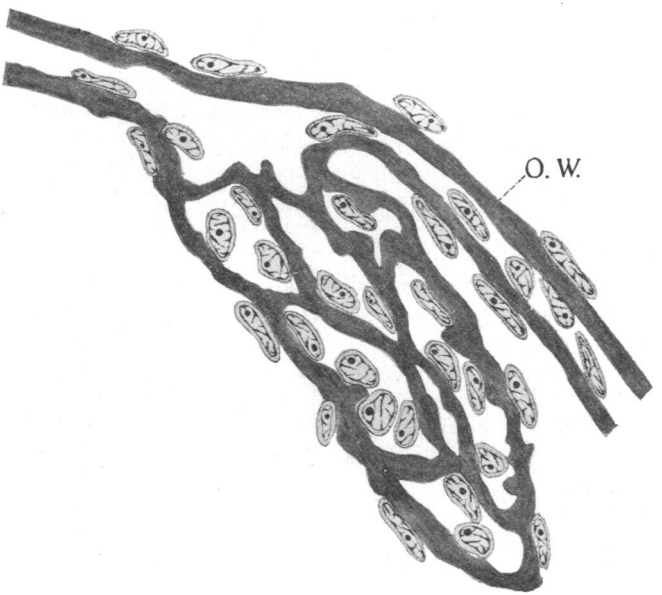

FIG. 6. A longitudinal section of a degenerating follicle sac taken from the ovary of a toad killed on the fifth of June. Zeiss apoc. 2 mm. Oc. 4.

much less folded than they were at an earlier period, and its cavity has nearly disappeared. Numerous cells are found closely applied to the walls of the sac at this time, but they show no degenerative changes, and are undoubtedly concerned in the absorption of the follicle sac itself. Much smaller structures of the same general character as that shown in Fig. 6 can be seen in the ovaries of toads killed the latter part of June; but after this time no traces of them can be found.

The follicle sacs, having served the important function of attaching the eggs to the walls of the ovary and of supplying them with nourishment during their growth period, are of

no further use after the eggs pass into the body cavity. They therefore undergo rapid degeneration and absorption to make room for the young eggs that are in the process of development.

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